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Thienopyridine ureas as dual inhibitors of the VEGF and Aurora kinase families

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ABSTRACT

In an effort to identify multi-targeted kinase inhibitors with a novel spectrum of kinase activity, a screen of Abbott proprietary KDR inhibitors against a broad panel of kinases was conducted and revealed a series of thienopyridine ureas with promising activity against the Aurora kinases. Modification of the diphenyl urea and C7 moiety of these compounds provided potent inhibitors with good pharmacokinetic profiles that were efficacious in mouse tumor models after oral dosing. Compound **2** (ABT-348) of this series is currently undergoing Phase I clinical trials in solid and hematological cancer populations.

The Aurora kinases are a family of serine/threonine kinases that mediate multiple events in cell division.[1] Humans have three Aurora kinases, A, B and C, that are differentially localized and mediate distinct functions during mitosis. Because the Aurora kinases play a key role in mitosis and are overexpressed in multiple human tumor lines, there has been considerable interest in developing Aurora kinase inhibitors as antitumor agents. A number of small-molecule pan-Aurora kinase inhibitors have been reported and there are several compounds currently in Phase I/II clinical trials for cancer.[2] Preclinically, it has been shown that pan-Aurora inhibitors reproduce the phenotype of Aurora B selective agents and it has been suggested that the clinical behavior of pan-Aurora inhibitors will resemble compounds selectively targeting Aurora B rather than Aurora A.[3]

The VEGF receptor family of RTKs, most notably VEGFR2 or KDR, mediates the biological function of vascular endothelial growth factor (VEGF) which is a regulator of vascular permeability and an inducer of endothelial cell proliferation, migration and survival. Accordingly, interruption of the KDR mediated signaling cascade can provide therapeutic benefit in human cancers as demonstrated by the FDA approval of the anti-VEGF antibody Avastin™ and four small molecule KDR kinase inhibitors.[4]

The established notion of inhibiting multiple kinases involved in tumor progression with a single small molecule[5] and the compelling antitumor effects elicited by multi-targeted KDR kinase inhibitors such as sunitinib[6] and linifanib[7] led us to screen Abbott proprietary KDR kinase inhibitors for activity against a broad panel of kinases. This screen revealed that the previously disclosed[8] thienopyridine urea series of KDR inhibitors possessed modest but promising Aurora B activity and it was believed that dual inhibitors of these two enzymes could have unique clinical applications.[9] Compound **1** from this series was a potent inhibitor of KDR, both enzymatically (IC_{50} = 9 nM)[10,11] and cellularly (IC_{50} = 32 nM),[12] and a weak inhibitor of Aurora B, enzymatically (IC_{50} = 487 nM)[10] and cellularly (IC_{50} = 4200 nM).[13,14] Herein is described the effort to increase the Aurora B inhibitory activity of this series as well as improve other properties including oral bioavailability and in vivo antitumor activity. Because established SAR indicated that substitution of the thienopyridine C7 position and urea terminal phenyl was allowed and could be varied while maintaining KDR inhibition, we focused on those two areas of the molecule. This work led to the discovery of inhibitor **2** (ABT-348), a potent inhibitor of Aurora B as well as the VEGF and PDGF families of receptor tyrosine kinases, which is currently in Phase I clinical trials in solid and hematological cancer populations (see Fig. 1).

The preparation of **2** is shown in Scheme 1 and exemplifies the general preparation of the 7-substituted thienopyridine ureas. Suzuki coupling of thienopyridine **3**[8] with *N*-Boc-protected 4-aniline

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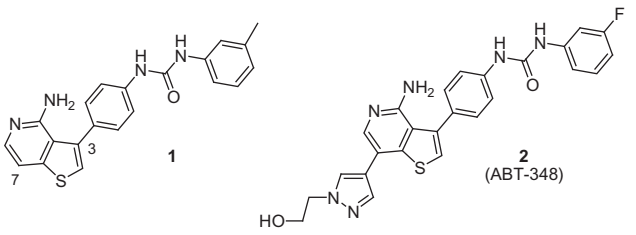
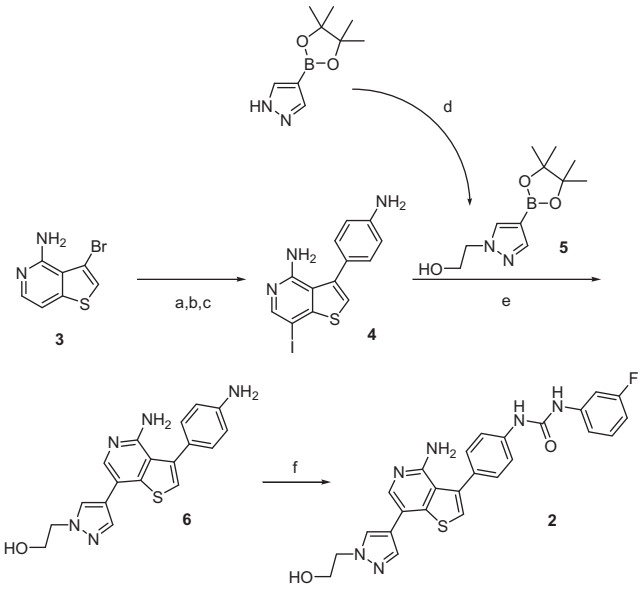


Figure 1. Thienopyridine urea kinase inhibitors.



Scheme 1. Reagents: (a) *tert*-Butyl 4-(4,4,5,5-tetramethyl-1,2,3-dioxaborolan-2-yl)phenylcarbamate, cat. $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 90 °C; (b) *N*-iodosuccinimide, DMF; (c) TFA, 70% (three steps); (d) ethylene carbonate, Cs_2CO_3, neat, 100 °C, 55%; (e) $PdCl_2$(dppf), Na_2CO_3, THF/MeOH/H_2O, 80 °C, 80%; (f) 3-fluorophenyl isocyanate, NMM, DMF, −20 °C, 80%.

boronate followed by selective C7 iodination and N-deprotection provided iodide **4**.[15] Suzuki coupling of **4** with *N*-(2-hydroxyethyl)pyrazole boronate **5**, prepared in one step from the N-unsubstituted pyrazole boronate, gave aniline **6**. Treatment of **6** with a small excess (1.05 equiv) of isocyanate at low temperature (−20 °C), to avoid bis-urea formation, provided compound **2** in 45% yield over the five linear steps. Iodide **4** was a versatile intermediate that could be coupled to a variety of alkenyl, alkynyl, aryl and heteroaryl boronate esters/boronic acids as well as carbon monoxide to give the corresponding methyl ester.

The prepared analogs were assessed for enzymatic activity against a panel of kinases including KDR, Aurora A and B, Flt-1, Flt-3, cKit, CSF1R and FGFR; compounds with sufficient KDR and Aurora B enzymatic activity (<50 nM) were examined for cellular activity in a KDR autophosphorylation assay and polyploidy induction assay (Aurora B activity). Inhibitors with potent cellular activity in both assays (<50 nM) were tested in an estradiol-induced mouse uterine edema model[11] which evaluated acute in vivo KDR activity after oral dosing. Selected inhibitors were then assessed in xenograft mouse tumor models with cancer cell lines such as HT1080 (human fibrosarcoma) and RS4;11 (human leukemia).

The effect of C7 substitution on enzymatic and cellular KDR and Aurora B inhibitory activity using the 3-methylphenyl urea is shown in Table 1. In general, a variety of C7 substituents provided analogs with potent KDR inhibition while potent Aurora B inhibition was more difficult to establish. For example, while alkenyl (**7**), alkynyl (**8**) and carboxamide (**9**) substitution provided potent inhibition of both KDR and Aurora B, phenyl (**10**) and phenyl bioisosteres including furyl (**11**) and thiophene (**12**) failed to give potent Aurora B inhibition. Examination of other heteroaryl substituents at this position revealed that while 3-pyrrole (**13**) and 3-pyrazole (**14**) gave somewhat better Aurora B enzymatic potency than other small heterocycles, the 4-pyrazole (**15** and **16**) was uniquely able to provide robust KDR and Aurora B activity.

The SAR of urea phenyl substitution using the (*N*-methylpyrazol-4-yl) moiety at C7 is shown in Table 2 which includes in vivo efficacy. It can be seen that a number of phenyl substituents provided inhibitors with excellent KDR and Aurora B enzymatic/cellular potency as well as potent oral activity. More specifically, 2-, 3- and 4-substitution (**17**, **16**, **18**) was allowed as was unsubstituted phenyl (**19**). The electronic nature of the substituents were not crucial for enzymatic activity as halides (**20**–**23**), methoxyl (**24**, **25**), and trifluoromethoxyl (**26**) substituents gave potent compounds; some electron-poor substituents (**27**) diminished cellular and in vivo potency. It can be seen that most inhibitors in Table 2 possessed potent activity (ED_{50} <10 mg/kg) in the UE model; for comparison, inhibitor **1** was inactive up to 30 mg/kg in this assay. Disubstitution of the urea terminal phenyl was also allowed (data not shown).

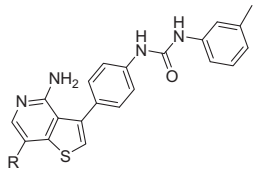
The effect of pyrazole substitution on KDR, Aurora B and UE activity is shown in Table 3. Consistent with the SAR shown in Table 1, many pyrazole substituents gave potent KDR inhibitors while Aurora B activity was less general. For example, while *N*-methyl was well tolerated, increasing the bulk of the pyrazole *N*-alkyl substituents adversely affected Aurora activity (compare **21**, **28**, **29** and **30**). However, if one or more heteroatoms were present in this substituent, Aurora activity, both enzymatic and cellular, was restored. This included sulfones (**31**), tertiary amines (**32**), amides (**33**), alcohols (**2**, **34**) or combinations of these moieties (**35**). It should be mentioned that attempts to improve the generally low aqueous solubility of these compounds with pyrazole substituents bearing tertiary amines (e.g., **32** and **35**) ultimately failed due to high clearance and low oral bioavailability in vivo as reflected in a lack of UE assay activity (e.g., **32**).

Modeling of thienopyridine **2** in an inactive conformation of Aurora B kinase was carried out to rationalize this SAR and the results are shown in Fig. 2a. In addition to the canonical hinge and back pocket interactions, the C7 substituent projects into a solvent-accessible extended-hinge region, that is flanked by hydrophobic residues Leu93 and Gly160 that narrow the available volume for substituents. This model agrees with the observation that large or branched substituents are less well accommodated, and that heteroatoms are adequately solvated. While the origin of the C7 substitution-based boost in Aurora B potency is not entirely clear, the pyrazole appears to optimally fit the 'extended-hinge' region of this enzyme as depicted in Fig. 2b.

Safety testing indicated that some analogs were potent inhibitors of Cyp3A4,[16] including time-dependent inhibition,[17] while others were devoid of this activity. In general, *para*-substitution of the diphenyl urea or increasing the bulk of the pyrazole substituent would decrease or eliminate Cyp3A4 inhibition altogether. For example, inhibitor **21** was a moderate inhibitor of Cyp3A4 (IC_{50} 7.9 μM) that was more potent after a 15 min preincubation (IC_{50} 2.7 μM) whereas analogs such as **25**, **28**, **34** and **2** had only weak activity (<20%) up to 10 μM (**2**, IC_{50} >30 μM) with no time dependency.

A summary of the mouse pharmacokinetic data for several inhibitors is shown in Table 4. In contrast to earlier compounds such as **1**, the C7 pyrazole-substituted analogs were generally characterized by improved half-lives, relatively low clearance and good

Table 1
SAR of C7 substitution



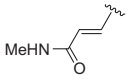
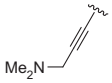
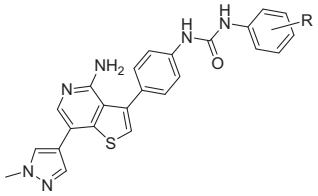
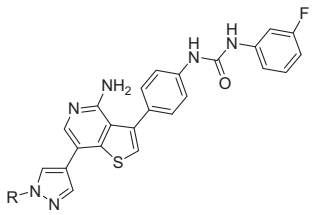
Compd	R	IC_{50} (nM)			EC_{15}[13] polyploidy (nM)
		KDR[10]	KDR_{cell}[12]	Aur B[10]	
1	H	9	32	487	4140
7		6	22	80	87
8		7	8	11	141
9		2	1	13	46
10	Phenyl	13	33	3280	Not tested
11		8	14	100	>300
12		9	31	179	>300
13		4	1	68	389
14		6	3	66	161
15		2	14	8	31
16		3	14	16	55

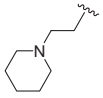
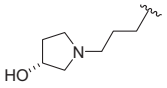
Table 2
SAR of urea terminal phenyl substitution



Compd	R	IC_{50} (nM)		EC_{15} (nM) Polyploidy[13]	ED_{50} (mg/kg, po) uterine edema[11]
		KDR[10]	Aur B[10]		
16	3-Me	3	16	55	1.0
17	2-Me	2	8	2	3.5
18	4-Me	6	50	64	1.0
19	H	3	6	9	0.3
20	2-F	3	18	<30	1.1
21	3-F	3	9	6	0.3
22	3-Cl	7	70	18	2.0
23	4-Cl	8	48	27	0.3
24	2-OMe	6	52	>100	8.0
25	4-OMe	6	114	57	1.3
26	4-OCF_3	3	17	12	2.6
27	3-$CONH_2$	2	23	413	>10

Table 3
SAR of pyrazole substitution



Compd	R	IC_{50} (nM)		EC_{15} (nM) polyploidy[13]	ED_{50} (mg/kg, po) uterine edema[11]
		KDR[10]	Aur B[10]		
21	Me	3	9	6	0.3
28	*n*-Pr	15	31	4	1.5
29	*i*-Bu	25	177	355	1.1
30	*i*-Pent	25	529	Not tested	Not tested
31		1	11	4	10
32		4	8	<3	>10
33		6	5	1	0.4
2		2	7	12	3.2
34		3	1	2	1.2
35		2	2	<1	Not tested

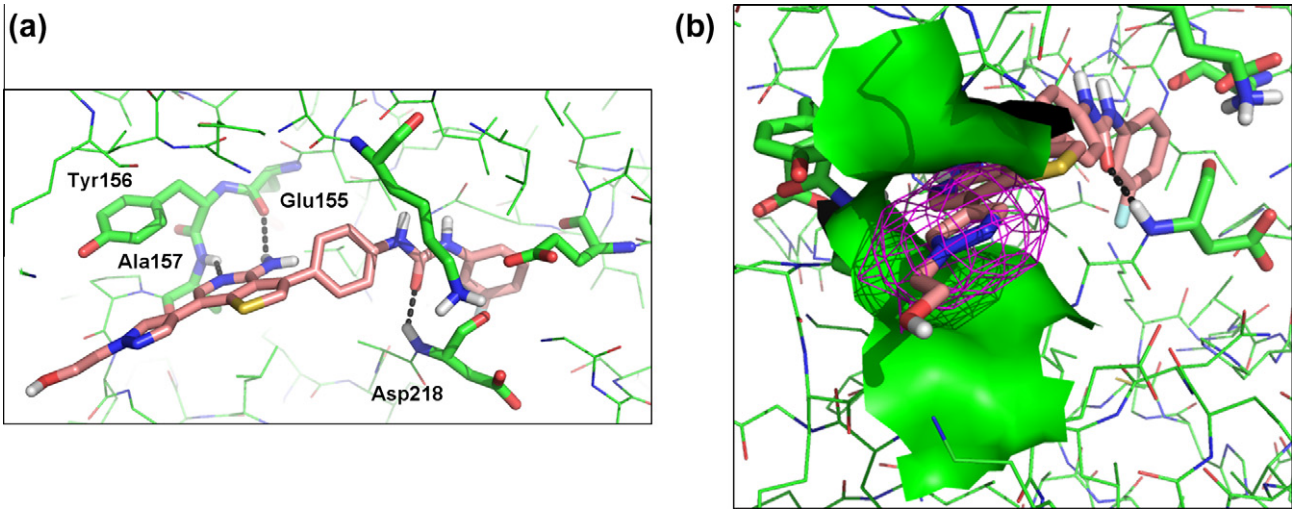


Figure 2. (a) Model of thienopyridine **2** bound to active site of Aurora B (inactive conformation) with hinge hydrogen bonds to Glu155 C=O and Ala157 N–H and urea hydrogen bond to Asp218 N–H. (b) *N*-(2-ethoxy)pyrazole moiety of **2** (wire-mesh surface) occupying the 'extended-hinge' region of Aurora B. Residues Leu93 (top) and Gly160 (bottom) form the surface (green) that flanks the pyrazole unit.

oral bioavailability. Consistent with the uterine edema screening data, these inhibitors provided significant exposure with oral dosing and were potent inhibitors of tumor growth in murine models. For example, compounds **16**, **21** and **28** demonstrated significant tumor growth inhibition (>70%) in the HT1080 model when dosed orally at 10 mg/kg, daily.

In general this series of compounds exhibited some degree of hERG blockade[18] as measured in a patch clamp assay and efforts to completely eliminate this activity while retaining the other required properties were unsuccessful. For example, compound **2** had an IC_{50} of 1 μM[19] in this assay. However, in the anesthetized dog, this analog prolonged QT by 11 ms at a plasma concentration

Table 4
Mouse pharmacokinetic data of selected compounds[a]

Compd	iv (3 mg/kg)			po (10 mg/kg) AUC (μg·h/mL)	*F* (%)
	$t_{1/2}$ (h)	V_D (L/kg)	Cl (L/h·kg)		
1	0.3	1.7	4.0	0.45	18
16	1.5	2.3	1.1	5.64	59
21	3.6	0.9	0.2	44.0	78
25	1.3	1.5	0.9	5.22	44
28	0.8	1.2	1.0	5.26	52
34	2.9	1.2	0.3	14.6	42
2	4.7	1.7	0.3	18.6	47

[a] PK studies in CD1(ICR) males.

Table 5
Kinase inhibition profile of **2**

Kinase	IC_{50}[a] (nM)	Kinase	IC_{50}[a] (nM)
KDR	4	FYN	110
Aur B	2	FGFR1	188
Aur A	12	ALK	363
Flt1	32	ROCK1	456
PDGFRβ	3	IGF1R	539
CSF1R	16	JAK2	>10,000
LCK	3	JAK3	>10,000
ABL	12	CDK9	>10,000
RET	7	GSK3a	>10,000

[a] TR-FRET assay.

of 32.1 μM and by 25 ms at 117 μM which represent 10- and 36-fold the predicted oral human C_{max} of approximately 3.7 μM.

After considering the efficacy, pharmacokinetic and safety profiles of all analogs, it was decided that inhibitor **2** would be taken through advanced preclinical evaluation. Evaluation of **2** for inhibitory activity across a panel of kinases revealed a unique kinome profile (Table 5), characterized by potent inhibition of the VEGFR, PDGFR and SRC families in addition to the Aurora inhibition.[20]

Compound **2** is lipophilic in nature (log*D* 4.5 at pH 7) with low aqueous solubility (<78 μM, physiological pH range), high permeability and extensive protein binding (>99%, all species tested). The pharmacokinetic profile is characterized by low plasma clearance (Clp <0.3 L/h·kg), moderate volumes of distribution (V_{ss} 0.4–1.2 L/kg) and half-lives of 4–5 h in mouse, rat and dog. Inhibitor **2** demonstrates significant antitumor efficacy in both solid and hematological xenograft models after intravenous, minipump or parenteral once-weekly dosing.[21]

In summary, a series of thienopyridine ureas with potent activity against both KDR and Aurora B has been identified. SAR work has provided analogs with significant cellular activity, favorable oral PK profiles in multiple species and robust antitumor activity in multiple preclinical models. Compound **2** from this series was advanced into clinical trials.

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